

APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

Claims 30, 44, 46 and 48 are amended as follows:

30. A method for assaying for a specific nucleic acid sequence that is within a target RNA, wherein said target RNA is a single-stranded RNA, said method comprising the following steps:

(1)I. ~~providing a single-stranded said target~~ RNA comprising said specific nucleic acid sequence;

(2)II. hybridizing said target RNA to a reagent (A), which is a single-stranded oligo nucleic acid complementary to a sequence 5' of, and adjacent to, the 5' end of said specific nucleic acid sequence that is within the target RNA, which allows the target RNA to be cut at the 5' end of the specific nucleic acid sequence by the action of a reagent (D), which is a ribonuclease that degrades RNA in a DNA-RNA double-strand;

(3)III. cutting the target RNA at the 5' end of the specific nucleic acid sequence with reagent D to give a product;

(4)IV. hybridizing to said product of step (3)III, a reagent (B), which is a first single-stranded oligo DNA primer complementary to a sequence at the 3' end of said specific nucleic acid sequence;

(5)V. extending said first single-stranded oligo DNA primer to the 5' end of the specific nucleic acid sequence with a reagent (C), which is an RNA-dependent DNA polymerase and

with a reagent (E), which is deoxynucleoside triphosphates, to form a DNA-RNA double-strand;

(6)VI. digesting the RNA strand of said DNA-RNA double-strand from step (5V) with the reagent (D), to give a single-stranded DNA complementary to said specific nucleic acid sequence;

(7)VII. hybridizing to said single-stranded DNA from step (6)(VI) a reagent (F) which is a second single-stranded oligo DNA primer having the following sequences, in the following order, beginning at the 5' end and proceeding in a 5' to 3' direction: i) a promoter sequence for a DNA-dependent RNA polymerase, ii) an enhancer sequence for said promoter sequence, and iii) a sequence at the 5' end of said specific nucleic acid sequence;

(8)VIII. extending said second oligo DNA primer to the 5' end of said single-stranded DNA with a reagent (G), which is a DNA-dependent DNA polymerase and with said reagent (E);

(9)IX. synthesizing a single-stranded RNA from said promoter sequence with a reagent (H), which is a DNA-dependent RNA polymerase and a reagent (I), which is ribonucleoside triphosphates;

(10)X. either:

(c) cycling said single-stranded RNA from step (9)(IX) to step (4)(IV), or

(d) hybridizing to said single-stranded RNA from step (9)(IX) a reagent (J), which is a single-stranded oligo DNA complementary to said specific nucleic acid sequence, labeled so that it gives off a measurable

fluorescent signal upon hybridization with a nucleic acid containing said specific nucleic acid sequence; and

~~(11)~~XI after addition of reagents (A) to (J), measuring at least once a fluorescent signal from said hybrid formed in step ~~(10)~~(X) (b);

wherein said reagents (A) to (J) are added to a reaction vessel one by one, in functional combinations, or all at once.

44. The method according to Claim 30, which further comprises a step of detecting or quantifying the ~~single-stranded~~target RNA in the sample based on the measured fluorescent signal or change in the measured fluorescent signal.

46. The method according to Claim 30, wherein prior to said step ~~(10)~~(X) (b) acetate is added as a reagent.

48. The method according to Claim 30, wherein prior to said step ~~(10)~~(X) (b) sorbitol is added as a reagent.